

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Canceled)
2. (Previously Presented) The method of Claim 46, further comprising washing the capture antibody:target molecule complex to remove unbound sample after step (a).
3. (Canceled)
4. (Previously Presented) The method of Claim 46, wherein the capture antibody is bound to a solid support or carrier during step (a) or (b).
5. (Previously Presented) The method of Claim 46, wherein the capture antibody is in solution during step (a) or (b).
- 6-7. (Canceled)
8. (Previously Presented) The method of Claim 46, wherein the target molecule is an organic compound having a molecular weight of about 100 to about 1000 grams/mole.
9. (Previously Presented) The method of Claim 46, wherein the target molecule is a protein or fragment thereof.
10. (Previously Presented) The method of Claim 9, wherein the protein is a cytokine selected from the group consisting of growth hormone, insulin-like growth factors, human growth hormone, N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxine, insulin, proinsulin, relaxin, pror relaxin, glycoprotein hormones, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), leutinizing hormone (LH),

hematopoietic growth factor, vesicular endothelial growth factor (VEGF), hepatic growth factor, fibroblast growth factor, prolactin, placental lactogen, tumor necrosis factor-alpha, tumor necrosis factor-beta, mullerian-inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, vascular endothelial growth factor, integrin, nerve growth factors (NGFs), NGF-beta, platelet-growth factor, transforming growth factors (TGFs), TGF-alpha, TGF-beta, insulin-like growth factor-I, insulin-like growth factor-II, erythropoietin (EPO), osteoinductive factors, interferons, interferon-alpha, interferon -beta, interferon-gamma, colony stimulating factors (CSFs), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF), thrombopoietin (TPO), interleukins (ILs), IL-1, IL-1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, LIF, SCF, neurturin (NTN) and kit-ligand (KL).

11. (Previously Presented) The method of Claim 46, wherein the sample is selected from the group consisting of blood, serum, sputum, urine, semen, cerebrospinal fluid, bronchial aspirate and organ tissue.

12. (Previously Presented) The method of Claim 5, wherein the capture antibody is labeled with biotin and is bound to a streptavidin or avidin labeled support.

13. (Previously Presented) The method of Claim 46, wherein the detectable non-primer probe comprises a nucleic acid having a fluorescent dye label.

14. (Original) The method of Claim 13, wherein the fluorescent dye label comprises two dyes, a reporter dye and a quencher dye, which fluoresce at different wavelengths.

15-16. (Canceled)

17. (Previously Presented) The method of Claim 46, wherein the nucleic acid detector molecule is RNA and the RNA detector molecule is reverse transcribed to form DNA before or during amplifying step d.

18. (Previously Presented) The method of Claim 46, wherein the RINA detector molecule is reversed transcribed at a temperature sufficient to dissociate the detector molecule from the capture antibody:target molecule:aptamer ternary complex and reverse transcribe the RNA.

19. (Original) The method of Claim 18, wherein the temperature is about 50C to about 70C.

20. (Original) The method of Claim 4, wherein the solid support is a PCR tube.

21-23. (Canceled)

24. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 1000 pg/mL.

25. (Previously Presented) The method of Claim 24, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 100 pg/mL.

26. (Previously Presented) The method of Claim 25, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 1 pg/mL.

27. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 100 to about 5000 pg/mL.

28. (Previously Presented) The method of Claim 27, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a

concentration of about 100 to about 1000 pg/mL.

29. (Previously Presented) The method of Claim 27, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 1000 to about 5000 pg/mL.

30. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 3 to about 5000 pg/mL.

31. (Previously Presented) The method of Claim 30, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 3 to about 1000 pg/mL.

32. (Previously Presented) The method of Claim 30, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 3 to about 100 pg/mL.

33. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.4 to about 5000 pg/mL.

34. (Previously Presented) The method of Claim 33, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.4 to about 1000 pg/mL.

35. (Previously Presented) The method of Claim 34, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.4 to about 100 pg/mL.

36. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 1 to about 5000 pg/mL.

37. (Previously Presented) The method of Claim 36, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 1 to about 1000 pg/mL.

38. (Previously Presented) The method of Claim 37, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 1 to about 100 pg/mL.

39. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 5000 pg/mL.

40. (Previously Presented) The method of Claim 39, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 1000 pg/mL.

41. (Previously Presented) The method of Claim 40, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 100 pg/mL.

42. (Previously Presented) The method of Claim 46, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.005 to about 5000 pg/mL.

43. (Previously Presented) The method of Claim 42, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.005 to about 1000 pg/mL.

44. (Previously Presented) The method of Claim 43, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.005 to about 100 pg/mL.

45. (Previously Presented) The method of Claim 44, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.005 to about 1 pg/mL.

46. (Previously Presented) A method for quantitating or detecting the presence of a target molecule in a sample which may contain the target molecule and a nuclease, comprising:

(a) exposing the sample which may contain the target molecule to a capture antibody or target molecule binding fragment thereof which binds to the target molecule under conditions whereby a capture antibody:target molecule or a target molecule binding fragment:target molecule complex is formed;

(b) adding to the complex from step (a), an RNA or DNA aptamer detector molecule which binds to the target molecule to form a capture antibody:target molecule:aptamer or a target molecule binding fragment:target molecule:aptamer ternary complex;

(c) washing the ternary complex from step (b) to remove said nuclease;

(d) when the aptamer is an RNA detector molecule, reverse transcribing the RNA to DNA;

(e) amplifying the DNA aptamer or DNA obtained by step (d) by PCR amplification;
and

(f) quantitating or detecting the PCR amplified DNA using a detectable non-primer

probe which binds to the DNA using real time PCR during PCR amplification;
wherein quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule.

47. (New) A method for quantitating or detecting the presence of a target molecule in a biological sample which may contain the target molecule and a nuclease, comprising:

(a) exposing the sample which may contain the target molecule to a capture antibody or target molecule binding fragment thereof which binds to the target molecule under conditions whereby a capture antibody:target molecule or a target molecule binding fragment:target molecule complex is formed;

(b) adding to the complex from step (a), an RNA or DNA aptamer detector molecule which binds to the target molecule to form a capture antibody:target molecule:aptamer or a target molecule binding fragment:target molecule:aptamer ternary complex;

(c) washing the ternary complex from step (b) to remove said nuclease;

(d) when the aptamer is an RNA detector molecule, reverse transcribing the RNA to DNA;

(e) amplifying the DNA aptamer or DNA obtained by step (d) by PCR amplification;
and

(f) quantitating or detecting the PCR amplified DNA using a detectable non-primer probe which binds to the DNA using real time PCR during PCR amplification;

wherein quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about .005 to about 5000 pg/mL.

48. (New) The method of Claim 47, further comprising washing the capture antibody:target molecule complex to remove unbound sample after step (a).

49. (New) The method of Claim 47, wherein the capture antibody is bound to a solid support or carrier during step (a) or (b).

50. (New) The method of Claim 47, wherein the capture antibody is in solution

during step (a) or (b).

51. (New) The method of Claim 47, wherein the target molecule is a protein or fragment thereof.

52. (New) The method of Claim 51, wherein the protein is a cytokine selected from the group consisting of growth hormone, insulin-like growth factors, human growth hormone, N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxine, insulin, proinsulin, relaxin, prorelaxin, glycoprotein hormones, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), leutinizing hormone (LH), hematopoietic growth factor, vesicular endothelial growth factor (VEGF), hepatic growth factor, fibroblast growth factor, prolactin, placental lactogen, tumor necrosis factor-alpha, tumor necrosis factor-beta, mullerian-inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, vascular endothelial growth factor, integrin, nerve growth factors (NGFs), NGF-beta, platelet-growth factor, transforming growth factors (TGFs), TGF-alpha, TGF-beta, insulin-like growth factor-I, insulin-like growth factor-II, erythropoietin (EPO), osteoinductive factors, interferons, interferon-alpha, interferon -beta, interferon-gamma, colony stimulating factors (CSFs), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF), thrombopoietin (TPO), interleukins (ILs), IL-1, IL-1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, LIF, SCF, neurturin (NTN) and kit-ligand (KL).

53. (New) The method of Claim 47, wherein the sample is selected from the group consisting of blood, serum, sputum, urine, semen, cerebrospinal fluid, bronchial aspirate and organ tissue.

54. (New) The method of Claim 50, wherein the capture antibody is labeled with biotin and is bound to a streptavidin or avidin labeled support.

55. (New) The method of Claim 47, wherein the detectable non-primer probe comprises a nucleic acid having a fluorescent dye label.

56. (New) The method of Claim 55, wherein the fluorescent dye label comprises two dyes, a reporter dye and a quencher dye, which fluoresce at different wavelengths.

57. (New) The method of Claim 47, wherein the nucleic acid detector molecule is RNA and the RNA detector molecule is reverse transcribed to form DNA before or during amplifying step d.

58. (New) The method of Claim 47, wherein the RNA detector molecule is reversed transcribed at a temperature sufficient to dissociate the detector molecule from the capture antibody:target molecule:aptamer ternary complex and reverse transcribe the RNA.

59. (New) The method of Claim 58, wherein the temperature is about 50C to about 70C.

60. (New) The method of Claim 49, wherein the solid support is a PCR tube.

61. (New) The method of Claim 47, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 1000 pg/mL.

62. (New) The method of Claim 60, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 100 pg/mL.

63. (New) The method of Claim 62, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration equal to or less than about 1 pg/mL.

64. (New) The method of Claim 47, wherein said quantitating or detecting the PCR

amplified DNA quantitates or detects the target molecule when present at a concentration of about 100 to about 5000 pg/mL.

65. (New) The method of Claim 47, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 3 to about 5000 pg/mL.

66. (New) The method of Claim 47, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.4 to about 5000 pg/mL.

67. (New) The method of Claim 47, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 5000 pg/mL.

68. (New) The method of Claim 67, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 1000 pg/mL.

69. (New) The method of Claim 68, wherein said quantitating or detecting the PCR amplified DNA quantitates or detects the target molecule when present at a concentration of about 0.03 to about 100 pg/mL.